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Product Information

Sigma-Aldrich Insoluble Enzymes

Product Description

Insoluble enzyme products are produced by reacting a conventional "soluble" enzyme with an inert base, to give an insoluble conjugate that retains the activity of the original enzyme. Applications are almost unlimited. By selecting the "inert" base carefully, a highly active "resin" or "gel" can be produced with which the enzyme reaction can be quickly catalyzed by momentary contact with the substrate in a suitable medium.[1] This can be carried out in a batch slurry, or even through a small column. In either case, the insoluble enzyme does not remain as a contaminant of the reaction mix. It can be readily filtered or centrifuged out of the batch slurry, or remain behind in the column. Many unstable enzymes seem to be much more stable in the insoluble form. In fact, with proper regeneration and storage, insoluble enzymes can be reused many, many times.

A highly popular choice of inert base is agarose.[2,3] Agarose is quite inert in most systems and allows high activities per unit weight. Several widely used enzymes conjugated to agarose beads are offered. Insoluble enzymes on polyacrylamide, acrylic beads, and carboxymethyl cellulose have also historically been prepared. Polyacrylamide, acrylic beads, and carboxymethyl cellulose matrices also have useful applications, but because of their absorptive properties, require more discreet handling.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses.

Preparation Instructions

Note: Buffer conditions may need to be adjusted to take into account the particular properties of the enzyme on the enzyme-conjugate. This is in order not to compromise the activity and functionality of the enzyme.

1. If the product is a lyophilized powder, suspend the required amount of resin at a concentration of 5-10 mg solid/mL water and allow brief hydration. Note: For polyacrylamide insoluble enzymes, it is necessary to allow the suspended enzyme to stand at 2-8 °C for two hours. Then proceed with the filter/wash step.

   Or

   If the product is a suspension, gently mix, then remove required amount for filter/wash step.

2. Filter and wash several times with water and/or buffer of choice to remove the suspension medium. Note: The packaging medium may contain stabilizers, which could inhibit enzyme activity.

3. Resuspend in the appropriate buffer. The enzyme is now ready for use.

Procedures

Assay Procedure (Batchwise)

1. Pipette a known aliquot of the prepared enzyme into a buffered substrate solution. Keep the suspension well-mixed during the reaction period.

2. After the appropriate interval of time, stop the reaction by removing the insoluble enzyme, either by filtering or by centrifuging.

3. Assay the clear supernatant fluid (or filtrate) for the extent of the reaction in the manner normally used for the soluble enzyme.

Assay Procedure (Column-wise)

1. Pour or pipette a known quantity of the prepared enzyme into a chromatographic column. For enzyme preparations which are very active, it is sometimes desirable to slurry the insoluble enzyme with an inert diluent to increase the bed volume of the packed enzyme. In the case of enzymes attached to agarose, use agarose beads as the inert diluent.

2. Drain off excess buffer. Equilibrate the column with ~1 bed volume of buffered reaction mixture which lacks one of the substrates.

3. Discard the equilibrating effluent.

4. Pass the buffered reaction mixture, complete with all of the essential reaction components, through the bed at a constant flow rate. If the temperature, substrate concentration, and enzyme are kept constant, the amount of conversion will be a function of the flow rate.

5. Assay the effluent for the extent of reaction as in the batchwise procedure.

Re-use of the Insoluble Enzyme

1. Wash the insoluble enzyme with water and/or buffer until free of substrates. It is now ready for another reaction cycle. It may be stored in this form for 2-8 °C.

2. If long-term storage (more than 2-3 days) is desired, return agarose enzymes to the suspension medium indicated on the label.

References

1. Datta, S. *et al*., *J. Biotech*., **3(1)**, 1-9 (2013).
2. Zucca, P. *et al.*, *Molecules*, **21(11)**, E1577 (2016).
3. Zdarta, J. *et al*., *Catalysts*, **8**, 92 (doi: 10.3390/catal8020092) (2018).

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